CONTRIBUTIONS TO THE MICROBIOLOGY OF AUSTRALIAN SOILS. I.

NUMBERS OF MICROORGANISMS IN SOIL, AND THEIR RELATION TO CERTAIN EXTERNAL FACTORS.

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(Three Text-figures.)

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Introduction and Methods.

Very little work has yet been devoted to a systematic study of the microflora of Australian soils, apart from the mainly physiological work by Greig-Smith (1910–18) on special groups of soil bacteria and protozoa, and two contributions by Dixon (1928–30) dealing almost only with the types of fungi occurring in Victorian soils. The present work represents an attempt to study the soil microflora on a broader basis, starting with an investigation of the numbers of bacteria, actinomycetes and fungi in soils of different types and in their relation to certain external factors, especially moisture and temperature.

Since it was desired to compare the numbers of microorganisms, the method of plate counting was used in spite of its serious limitations (especially the fact that its results represent only a fraction of the total soil microflora), because this method is the only one yet capable of giving numerical expressions for the soil fungi and actinomycetes. Bacteria and actinomycetes were counted on the following agar medium: dextrose 2·0 gm.; casein, dissolved in 0·1n NaOH, 0·2 gm.; K_2HPO_4 0·5 gm.; $MgSO_4$ 0·2 gm.; agar 20·0 gm.; H_2O 1,000 c.c.; pH 6·5–6·6. Dilutions of 1 : 50,000 to 1 : 250,000 were used, according to the character of the soil, and 4 to 5 parallel plates were incubated for 7–8 days at 28°C.* Fungi were counted on an agar medium of the following composition: dextrose 10·0 gm.; asparagine 1·0 gm.; KH_2PO_4 2·0 gm.; $MgSO_4$ 0·5 gm.; NaCl 0·5 gm.; agar 25·0 gm.; H_2O 1,000 c.c.; pH 4·6–4·8. Dilutions of 1 : 4,000 to 1 : 20,000 were used, and 4 to 5 parallel plates were incubated for 4–5 days at 28°C. The final dilutions for both platings were made up from the same soil suspension, prepared by shaking 20 grams of fresh soil for 4 minutes with 200 c.c. of sterile tap water. The total amount of data was tested for the χ^2 distribution, according to Fisher (1930): if the plate counts have given a reliable picture of the density of microorganisms counted, the index of dispersion, χ^2,† shall be distributed in a known manner, and if one, as here, is dealing with sets with a variable number of parallel plates,

* This somewhat high temperature of incubation was chosen because of the difficulty of keeping a lower constant temperature during the summer months.

† Calculated by the formula : $\chi^2 = \frac{S(x-\bar{x})^2}{\bar{x}}$, where x is the number of colonies counted on each plate, $\bar{x}$ the mean, $S(x-\bar{x})^2$ the sum of the squares of the deviations from $\bar{x}$.

the difference $2S(\chi^2) - 2S(n) - 1$ (where $S(\chi^2)$ is the sum-total of all the values of χ^2, n the number of parallel plates minus 1, and S(n) the sum-total of all the values of n) shall not materially exceed 2·0. The following results were found:

Bacteria.	Actinomycetes.	Fungi.
$S(\chi^2)$: 346·23	$S(\chi^2)$: 279·0	$S(\chi^2)$: 285·21
S(n) : 342	S(n) : 342	S(n) : 327
Difference : 0·18	Difference : − 2·51	Difference : − 1·67

While the result for the bacteria is entirely normal, there is, in the case of the actinomycetes, a tendency to subnormality (cf. Jensen, 1931*b*), but since the difference is not very great, we may be justified in regarding the majority of the actinomyces-counts as reliable. The difference for the fungi is within the permissible limits, and the technique must therefore be considered as giving a reliable index of the numbers of mycelial fragments and fungous spores capable of developing on the medium used.

The reaction of the soils was measured colorimetrically. Water content was determined by drying at 96–98°C. Organic matter was determined by ignition, and the water-holding capacity by the simple method described by Christensen (1923); neither of these two methods is very accurate, but their results may be used for comparative purposes, as was the main consideration in this case.

A. *Numbers of Microorganisms in Various Soils from New South Wales.*

The following 50 soils were examined:

1. Light sand soil, poor in humus, grass-covered, under bushes, Cooper Park, Sydney.
2. Heavy loam, rich in humus, flower-bed, Sydney University.
3.* Sand-mixed humus soil, Glen Innes, N.S.W.
4.* Red clay, poor in humus, Griffith, N.S.W.
5.* Red sandy loam, poor in humus, Cowra, N.S.W.
6.* Alluvial clay, fairly rich in humus, Bathurst, N.S.W.
7.* Red-brown loam, poor in humus, Griffith, N.S.W.
8. Heavy loam, rich in humus, flower-bed, Sydney University.
9.* Red loam, poor in humus, Griffith, N.S.W.
10.* Red loam, poor in humus, Griffith, N.S.W.
11. Heavy loam, very rich in humus, from pot experiments, School of Agriculture, Sydney University.
12.* Light sand soil, poor in humus, Goulburn, N.S.W.
13.* Sandy loam, very rich in humus, Scone, N.S.W.
14.* Red sandy loam, poor in organic matter, Temora, N.S.W.
15. Coarse, dark sand, very poor in humus, Rose Bay Heights, Sydney.
16.* Light sandy loam, poor in organic matter, Goulburn, N.S.W.
17.* Red loam, fairly rich in humus, Griffith, N.S.W.
18.* Light loam, fairly rich in humus, lucerne field.
19.* Light, coarse, gravelly sand, poor in humus, Bathurst, N.S.W.
20. Light loam, rich in humus, sheep pen, McMaster Laboratory, Sydney University.
21. White sand, very poor in humus, grass-covered, Bellevue Hill, Sydney.
22. Clay, rich in humus, almost bare, under trees, Sydney University.
23. Clay, very rich in humus, under trees, Sydney University.
24. Light loam (garden), fairly rich in humus, North Sydney.
25. Sand, very poor in humus, under bushes, Rose Bay Heights, Sydney.
26. Sand, rich in humus, grass-covered, Rose Bay Park, Sydney.
27. Heavy loam, rich in humus, wheat field, St. John's College, Sydney University.
28. Dark sand, fairly rich in humus, under casuarinas, Watson's Bay, Sydney.
29. Clay, poor in humus, Glenfield, N.S.W.
30. Clay, poor in humus, Glenfield, N.S.W.
31. Heavy loam (garden), rich in humus, Richmond, N.S.W.
32. Light loam, garden, very rich in humus, Richmond, N.S.W.
33. Sand, poor in humus, grass-covered, Bellevue Hill, Sydney.
34. Loam, rich in humus, from bush, Wahroonga, N.S.W.

35. Coarse sand, poor in humus, grass-covered, Centennial Park, Sydney.
36. Dark sand, poor in humus, grass-covered, Bronte, Sydney.
37. Dark sand, poor in humus, under casuarinas, Manly, N.S.W.
38. Sandy loam, poor in humus, St. Leonard's Park, North Sydney.
39. Red-brown loam, fairly rich in humus, pasture, Hinchinbrook, N.S.W.
40. Black loam, rich in humus, pasture, Hinchinbrook, N.S.W.
41. Coarse sand, rich in humus, under eucalypts, Ryde, N.S.W.
42. Light loam, rich in humus, grass-covered, Fivedock, Sydney.
43. Light sand, poor in humus, grass-covered, Coogee, Sydney.
44. Dark sand, poor in humus, under bushes, Cooper Park, Sydney.
45. Sand, poor in humus, under ferns, bush, Longueville, N.S.W.
46. Heavy loam, rich in humus, flower-bed, Victoria Park, Sydney.
47. Sand, very poor in humus, under thin grass, Maroubra, Sydney.
48. Heavy loam, very rich in humus, clover field, Ryde, N.S.W.
49. Coarse, dark sand, fairly rich in humus, under heavy grass, Centennial Park, Sydney.
50. Light sand, poor in humus, bush, Wahroonga, N.S.W.

The soils marked * were obtained from the collection of soil samples in the School of Agriculture, Sydney University, through the kindness of Mr. G. Wright, lecturer in agricultural chemistry; these samples were air-dry, and were therefore, prior to examination, kept moist in the laboratory for 15–20 days in order to enable the microflora to reach a certain equilibrium. The others were freshly taken samples from the upper 10 cm. of the soil.

The numbers of microorganisms, together with the pH-values and the contents of water and organic matter, are recorded in Table 1, and the various correlation coefficients which have been calculated from these data in Table 2.

The numbers of bacteria vary within wide limits, from 0·3 to 95 millions per gm. of soil, and are generally similar to those recorded from other parts of the world (Waksman, 1916, 1922; Cutler, Crump and Sandon, 1923; Smith and Worden, 1925; Jensen, 1931*a*). The numbers show a very good correlation with the content of organic matter in the soils; the correlation coefficient amounts to no less than 0·806 (Table 2), which value is not reduced very much when we eliminate the influences of hydrogen-ion concentration and moisture by calculating the corresponding partial correlation coefficients (Fisher, 1930). The bacterial numbers, on the other hand, do not show any significant correlation with the hydrogen-ion concentration,* or with the relative moisture content if the influence of organic matter is eliminated; it is to be noted, though, that this is true only when soils of different character are compared, since periodical counts of bacteria in one and the same soil have revealed a very definite correlation between moisture and bacterial numbers, as will be shown later. We must therefore conclude that the quantity of organic matter is the most important among the factors considered here in determining the numbers of bacteria which are able to develop on agar plates. The disagreement of this result with what was previously observed in Danish soils (Jensen, 1931*a*) is probably due to the fact that the latter series included several typical peat soils, poor in microorganisms but very rich in inert organic matter, a soil type that is not represented in the present investigation. A somewhat abnormal picture is shown by some coarse, acid sand soils, generally poor in humus, from the neighbourhood of Sydney, which all (Nos. 15, 25, 28, 37, 41, 47, 50) have shown bacterial numbers very much lower (0·3–4·2 mill. per gm.) than other soils with corresponding reaction and humus content. This paucity of organisms applies also to the actinomycetes, but not to the fungi. There do not seem to be any notable differences between bacterial numbers in cultivated

* It is of importance to note that the calculations are based on the actual hydrogen-ion concentration, and not on the pH-values.

TABLE 1.
Counts of Microorganisms in 50 Soils of Different Character.

No.	Moisture.		pH.	Organic Matter %.	Bact.[2]	Act.[2]	Fungi.[3]	Ratio A. : B.
	H_2O %.	Degree.[1]						
1	8·3	26·8	4·6	4·5	2·7	3·9	312	1·45
2	21·6	49·1	5·5	13·8	14·8	3·8	160	0·26
3	32·8	56·6	5·3	24·4	95·6	26·1	724	0·36
4	19·3	59·4	6·8	6·1	16·7	3·2	8	0·19
5	10·7	36·0	5·1	2·6	5·6	2·0	29	0·35
6	20·8	53·3	5·2	7·5	11·9	2·7	66	0·23
7	12·9	40·3	5·7	5·0	8·7	2·8	67	0·32
8	21·8	56·6	7·3	13·8	41·6	3·7	120	0·09
9	19·6	50·3	6·1	7·2	13·7	1·9	62	0·13
10	13·3	41·6	6·0	4·6	20·8	10·9	28	0·52
11	26·7	47·7	4·7	16·1	43·8	2·7	451	0·07
12	18·4	43·8	4·9	4·4	29·5	4·8	553	0·16
13	25·1	52·3	6·0	17·4	43·1	35·7	394	0·83
14	10·9	34·1	5·2	3·5	2·5	2·2	68	0·86
15	12·2	70·2	4·8	1·9	0·8	0·3	265	0·35
16	15·1	50·5	4·5	5·0	6·0	10·5	745	1·75
17	19·2	50·6	7·9	10·3	12·7	22·0	24	1·73
18	22·0	57·9	4·9	8·2	30·1	10·3	361	0·34
19	11·7	52·0	5·1	1·4	6·4	3·4	87	0·53
20	19·0	42·2	7·2	12·3	26·8	7·4	114	0·28
21	7·9	33·6	5·1	1·0	6·9	1·8	231	0·26
22	20·9	62·4	6·1	12·1	40·4	5·9	146	0·15
23	32·0	68·8	6·5	19·0	53·2	7·1	664	0·13
24	10·1	27·3	6·9	7·4	21·9	3·0	70	0·14
25	6·2	24·3	5·6	1·6	1·0	1·0	112	0·98
26	22·3	51·8	6·1	10·9	25·9	10·7	310	0·42
27	26·5	64·6	5·6	10·8	16·1	8·0	258	0·50
28	16·8	47·3	5·2	7·4	1·1	0·1	241	0·10
29	19·1	54·6	6·1	6·6	2·7	3·4	65	1·25
30	17·7	49·8	5·9	6·4	9·8	5·8	202	0·59
31	18·4	51·1	6·0	11·6	18·0	4·9	254	0·27
32	24·0	61·5	6·3	16·1	57·3	11·1	350	0·51
33	10·6	34·7	6·3	2·9	4·1	3·7	144	0·90
34	25·0	62·5	5·8	12·6	14·0	3·8	300	0·27
35	18·5	52·1	5·9	4·7	9·4	3·5	131	0·37
36	11·2	32·2	6·4	5·8	10·6	7·8	236	0·73
37	15·3	41·9	5·2	4·8	0·6	0·2	280	0·27
38	8·4	23·6	5·3	6·8	3·5	4·0	227	1·13
39	18·9	45·5	5·4	9·1	19·4	6·8	417	0·35
40	23·6	54·9	5·7	10·4	24·2	9·2	163	0·33
41	18·6	48·3	5·4	11·9	3·3	1·6	403	0·50
42	15·2	38·0	6·6	11·0	30·6	8·7	322	0·28
43	8·3	28·1	5·6	2·6	3·6	3·0	191	0·83
44	13·5	42·2	5·3	4·6	11·2	1·4	284	0·13
45	10·8	31·7	5·5	5·8	7·3	1·8	99	0·24
46	16·8	42·0	7·5	9·8	33·6	4·5	151	0·13
47	4·5	18·2	4·5	1·7	0·3	0·2	98	0·67
48	29·7	64·5	5·7	16·9	49·0	7·0	1088	0·14
49	36·6	89·3	6·0	8·5	55·1	1·1	179	0·02
50	10·6	32·1	5·5	5·8	1·5	0·3	469	0·22

[1] H_2O in % of water-holding capacity.
[2] Millions per gm. of air-dry soil.
[3] Thousands per gm. of air-dry soil.

TABLE 2.

Correlation Coefficients from Counts of Microorganisms in Table 1.

Correlation between	r*	n*	P*	Significance.
Bacteria and Organic Matter, total	0·806	48	<0·01	Positive.
Same, partial, with elimination of:				
(*a*) Hydrogen-Ion Concentration	0·795	47	<0·01	—
(*b*) Degree of Moisture	0·742	47	<0·01	—
(*a*) and (*b*)	0·794	46	<0·01	—
Bacteria and Hydrogen-Ion Concentration, total	−0·211	48	>0·1	Negative.
Same, partial, with elimination of:				
(*a*) Organic Matter	0·054	47	>0·1	—
(*b*) Degree of Moisture	−0·115	47	>0·1	—
(*a*) and (*b*)	0·056	46	>0·1	—
Bacteria and Degree of Moisture, total	0·520	48	<0·01	Positive.
Same, partial, with elimination of:				
(*a*) Organic Matter	0·256	47	0·1–0·05	Negative.
(*b*) Hydrogen-Ion Concentration	0·496	47	<0·01	Positive.
(*a*) and (*b*)	−0·128	46	>0·1	Negative.
Actinomycetes and Organic Matter, total	0·585	48	<0·01	Positive.
Same, partial, with elimination of:				
(*a*) Hydrogen-Ion Concentration	0·576	47	<0·01	—
(*b*) Degree of Moisture	0·561	47	<0·01	—
(*a*) and (*b*)	0·556	46	<0·01	—
Actinomycetes and Hydrogen-Ion Concentration, total	−0·133	48	>0·1	Negative.
Same, partial, with elimination of:				
(*a*) Organic Matter	0·041	47	>0·1	—
(*b*) Degree of Moisture	−0·095	47	>0·1	—
(*a*) and (*b*)	0·026	46	>0·1	—
Actinomycetes and Degree of Moisture, total	0·210	48	>0·1	Negative.
Same, partial, with elimination of:				
(*a*) Organic Matter	−0·100	47	>0·1	—
(*b*) Hydrogen-Ion Concentration	0·189	47	>0·1	—
(*a*) and (*b*)	−0·093	46	>0·1	—
Bacteria and Actinomycetes, total	0·563	48	<0·01	Positive.
Same, partial, with elimination of:				
(*a*) Organic matter	0·192	47	>0·1	Negative.
(*b*) Hydrogen-Ion Concentration	0·552	47	<0·01	Positive.
(*c*) Degree of Moisture	0·544	47	<0·01	—
(*a*) and (*b*)	0·195	46	>0·1	Negative.
(*a*) and (*c*)	0·218	46	>0·1	—
Fungi and Organic Matter, total	0·543	48	<0·01	Positive.
Same, partial, with elimination of:				
(*a*) Hydrogen-Ion Concentration	0·678	47	<0·01	—
(*b*) Degree of Moisture	0·488	47	<0·01	—
(*a*) and (*b*)	0·578	46	<0·01	—
Fungi and Hydrogen-Ion Concentration, total	0·199	48	>0·1	Negative.
Same, partial, with elimination of:				
(*a*) Organic Matter	0·542	47	<0·01	Positive.
(*b*) Degree of Moisture	0·276	47	0·1–0·05	Negative.
(*a*) and (*b*)	0·440	46	<0·01	Positive.

TABLE 2.—*Continued.*

Correlation Coefficients from Counts of Microorganisms in Table 1.—Continued.

Correlation between	r*	n*	P*	Significance.
Fungi and Degree of Moisture, total	0·272	48	0·1–0·05	Negative.
Same, partial, with elimination of:				
(*a*) Organic Matter	−0·014	47	>0·1	—
(*b*) Hydrogen-Ion Concentration	0·331	47	0·05–0·02	Positive.
(*a*) and (*b*)	0·042	46	>0·1	Negative.
Bacteria and Fungi, total	0·525	48	<0·01	Positive.
Same, partial, with elimination of:				
(*a*) Organic Matter	0·175	47	>0·1	Negative.
(*b*) Hydrogen-Ion Concentration	0·581	47	<0·01	Positive.
(*a*) and (*b*)	0·094	46	>0·1	Negative.
Actinomycetes and Fungi, total	0·373	48	<0·01	Positive.
Same, partial, with elimination of:				
(*a*) Organic Matter	0·083	47	>0·1	Negative.
(*b*) Hydrogen-Ion Concentration	0·422	47	<0·01	Positive.
(*a*) and (*b*)	0·052	46	>0·1	Negative.
Ratio of Actinomycetes to Bacteria, and Organic Matter, total	−0·252	48	0·1–0·05	Negative.
Same, partial, with elimination of:				
(*a*) Hydrogen-Ion Concentration	−0·224	47	>0·1	—
(*b*) Degree of Moisture	−0·091	47	>0·1	—
(*a*) and (*b*)	−0·079	46	>0·1	—
Ratio A.: B. and Hydrogen-Ion Concentration, total	0·141	48	>0·1	Negative.
Same, partial, with elimination of:				
(*a*) Organic Matter	0·075	47	>0·1	—
(*b*) Degree of Moisture	0·065	47	>0·1	—
(*a*) and (*b*)	0·047	46	>0·1	—
Ratio A.: B. and Degree of Moisture, total	−0·371	48	<0·01	Positive.
Same, partial, with elimination of:				
(*a*) Organic Matter	−0·295	47	0·05–0·02	—
(*b*) Hydrogen-Ion Concentration	−0·352	47	0·02–0·01	—
(*a*) and (*b*)	−0·271	46	0·1–0·05	Negative.
Ratio of Fungi to Bacteria plus Actinomycetes, and Organic Matter, total	−0·279	48	Near 0·05	Doubtful.
Same, partial, with elimination of:				
(*a*) Hydrogen-Ion Concentration	−0·202	47	>0·1	Negative.
(*b*) Degree of Moisture	−0·226	47	>0·1	—
(*a*) and (*b*)	−0·173	46	>0·1	—
Ratio F.: B.+A. and Hydrogen-Ion Concentration, total	0·345	48	0·02–0·01	Positive.
Same, partial, with elimination of:				
(*a*) Organic Matter	0·289	47	Near 0·05	Doubtful.
(*b*) Degree of Moisture	0·319	47	0·05–0·02	Positive.
(*a*) and (*b*)	0·286	46	Near 0·05	Doubtful.
Ratio F.: B.+A. and Degree of Moisture, total	−0·176	48	>0·1	Negative.
Same, partial, with elimination of:				
(*a*) Organic Matter	−0·049	47	>0·1	—
(*b*) Hydrogen-Ion Concentration	−0·108	47	>0·1	—
(*a*) and (*b*)	−0·018	46	>0·1	—

TABLE 2.—*Continued.*

Correlation Coefficients from Counts of Microorganisms in Table 1.—Continued.

Correlation between	r*	n*	P*	Significance.
Ratio F.: B.+A. and Organic Matter, 7 abnormal soils excluded, total	−0·304	41	Near 0·05	Doubtful.
Same, partial, with elimination of Hydrogen-Ion Concentration	−0·245	40	>0·1	Negative.
Ratio F.: B.+A. and Hydrogen-Ion Concentration, 7 soils excluded, total	0·607	41	<0·01	Positive.
Same, partial, with elimination of Organic Matter	0·587	40	<0·01	—
Organic Matter and Hydrogen-Ion Concentration, total	−0·282	48	Near 0·05	Doubtful.
Same, partial, with elimination of Degree of Moisture	−0·204	47	>0·1	Negative.
Organic Matter and Degree of Moisture, total	0·480	48	<0·01	Positive.
Same, partial, with elimination of Hydrogen-Ion Concentration	0·446	47	<0·01	—
Hydrogen-Ion Concentration and Degree of Moisture, total	−0·222	48	>0·1	Negative.
Same, partial, with elimination of Organic Matter	−0·092	47	>0·1	—
Organic Matter and Hydrogen-Ion Concentration, 7 abnormal soils excluded, total	−0·186	41	>0·1	Negative.

* r is the correlation coefficient, n the number of observations minus 2 and, in case of partial correlations, minus the number of eliminated variates, P (from Table V.A., Fisher, 1930) the probability of the corresponding correlation coefficient being due to random sampling from an uncorrelated population; if the value of P exceeds 0·05, the correlation is not to be regarded as significant. (Fisher, 1930.)

and uncultivated soils, except so far as the former are generally richer in organic matter.

The numbers of actinomycetes vary within equally wide limits, from 0·1 to 36 mill. per gm. Like the bacteria, their numbers show a definite positive correlation with the content of organic matter, although not so pronounced, the correlation coefficient amounting to 0·585; its value is not reduced materially by eliminating the influences of hydrogen-ion concentration and moisture, with which two factors the numbers of actinomycetes do not show any significant correlation. Neither is there, when organic matter is eliminated, any significant correlation between numbers of bacteria and of actinomycetes, so that these two groups of organisms do not, *per se*, seem to have any influence upon each other, at least when different soils are compared (cf. below).

The ratio of actinomycetes to bacteria varies widely, from 0·065 (No. 11) to 1·75 (No. 16), without showing any significant correlation with the organic matter or the hydrogen-ion concentration. There is some indication of a negative correlation with the moisture-degree, although the correlation coefficient is reduced below significance when organic matter and hydrogen-ion concentration are both eliminated; a larger number of observations might have shown a significant result, but still this factor does not seem very important. Countings from the same soil at different contents of moisture give a quite different result, as will be shown below.

The numbers of fungi vary within still wider limits (from 8,000 to 1,088,000 per gm.) and are generally somewhat higher than previously found with a similar

technique in Danish soils (Jensen, 1931*a*). Their correlation with the organic matter content is definitely significant ($r = 0{\cdot}543$), and remains so when hydrogen-ion concentration and moisture are eliminated. There is no significant correlation with the degree of moisture, but the correlation with hydrogen-ion concentration shows interesting relationships: the total correlation is insignificant, but the partial correlation coefficient with elimination of organic matter is highly significant, also if the influence of moisture is eliminated too. In other words, although increases in organic matter as well as increase in acidity tend to increase the numbers of fungi, the former factor seems to be the more important, to judge by the correlation coefficients. It is also noteworthy that, although there is no general correlation between degree of moisture and fungal numbers, some soils from districts with low annual rainfall (Nos. 4, 5, 9, 10, 14 and 17) have shown the lowest numbers of all.

No real analysis of the composition of the fungous flora has been attempted, but it was noted that the predominant forms were species of *Penicillium*, *Mucor*, *Aspergillus*, *Trichoderma*, and *Fusarium* (cf. Dixon, 1928–30)—organisms that represent the bulk of soil fungi in most geographical regions. The genus *Zygorhynchus*, which, according to Waksman (1932), has the widest geographical distribution of all soil fungi, was observed only in one soil (No. 30). Neither has it been recorded by Dixon (1928–30); since it is an easily recognizable organism, it does not seem to be common in Australian soils. The aspergilli were present in most samples, contrary to what was the case in Danish soils (Jensen, 1931*a*); this agrees with the well-known preference of these fungi for high temperatures. In one soil (No. 9) they accounted for no less than 85% of the total number of fungal colonies, otherwise their numbers were usually quite low.

The ratio of fungi to bacteria plus actinomycetes did not show any significant correlation with the organic matter or moisture, and the correlation with hydrogen-ion concentration was quite low or even doubtful, contrary to what was observed in Danish soils (Jensen, 1931*a*), but if we exclude the 7 soils with abnormally low numbers of bacteria and actinomycetes, referred to above, from the calculation, we find a correlation coefficient of high significance, viz., 0·607, and when organic matter is eliminated, 0·587. This correlation is not so marked as in the Danish soils, but the range of hydrogen-ion concentration is also considerably narrower here—pH 4·5 to 7·9 against pH 3·3 to 8·4. The reaction seems thus generally to be a very important factor in determining the balance between fungi and bacteria plus actinomycetes, rather than the actual numbers of organisms.

B. *Periodical Counts of Microorganisms.*

The data which we have just discussed suffer from the disadvantage that we are here comparing soils of different character, with different physical structure, and containing organic matter and other microbial food of probably widely differing quality, and moreover, since the observations cannot all be carried out simultaneously, seasonal changes in the microflora may take place. It is therefore of interest to compare them with periodical counts of microorganisms in one and the same soil, in order to get an idea of the changes which the microflora may undergo according to changes in moisture and temperature, and of the possible existence of seasonal changes or spontaneous fluctuations in the numbers of bacteria. For this purpose, a plot of uncultivated, grass-covered soil was selected on the grounds of Sydney University; the soil was a heavy loam, rich in organic matter, of pH 5·4–5·5. Fifty counts of bacteria and actinomycetes and 45 counts of fungi were carried out in two periods, with weekly or bi-weekly intervals. The samples

were taken from the upper 10 cm. of soil within an area of 4 m.², each sample being a composite of 6 individual samples, scattered as evenly as possible over the sampled area. Three parallel samples were examined in order to test the uniformity of the soil; the following results were obtained:

No.	Average number of colonies, and standard deviation.			H_2O %.
	Bacteria.	Actinomycetes.	Fungi.	
I	55·0±6·83	24·5±3·51	24·8±2·50	29·5
II	61·0±2·16	25·3±3·40	31·0±4·32	29·5
III	57·0±1·83	23·8±2·87	28·3±4·99	29·6

(Dilution: Fungi, 1 : 20,000. Bacteria and Actinomycetes, 1 : 200,000. 4 parallel plates.)

There are here significant differences between numbers of fungi in samples I and II, and of bacteria in samples II and III, but since they are only small, we may consider that the heterogeneity of the soil accounts for only a minor part of the changes in the numbers of microorganisms; it may, for instance, have something to do with the divergence of bacterial and fungal numbers at approximately equal degree of moisture, with which these numbers are as a whole closely correlated, as will be shown below.

All samples were not taken at the same hour of the day, and another experiment was therefore carried out to test whether the numbers of microorganisms, especially bacteria, on the medium employed here showed any spontaneous fluctuations within short intervals of time, as demonstrated by Cutler, Crump and Sandon (1923) and later by Thornton and Gray (1930). Platings from 4 samples taken at 2-hour intervals yielded the following result:

No.	Time.	Soil Temp.	H_2O %.	Average number of colonies, and standard deviation.	
				Bacteria.	Actinomycetes.
I	10 a.m.	24°C.	26·2	47·0±4·85	20·6±4·56
II	noon	26°C.	25·9	54·3±8·46	21·3±6·19
III	2 p.m.	26°C.	26·9	52·2±7·16	26·0±6·75
IV	4 p.m.	25°C.	27·2	52·3±3·40	22·5±2·38

(Dilution 1 : 200,000; 4 parallel plates.)

There are no significant changes in the numbers of either bacteria or actinomycetes from 10 a.m. to 4 p.m., between which hours all samples were taken. The reason is probably that the dextrose-casein-agar is less selective and gives higher counts than the mannite-asparagine-agar used by Thornton and Gray (Jensen, 1931*b*; cf. also Smith and Worden, 1925, and Thornton and Fisher, 1927). Since our medium thus does not show the spontaneous fluctuations, it would seem well adapted for studying the changes in the numbers of bacteria over longer periods in relation to external factors.

TABLE 3.

Periodical Counts of Microorganisms.

No.	Date.	Temp. °F.[1]	H_2O %[2].	Bact.[3]	Actinomyc.[3]	Fungi.[4]	Ratio A. : B.
1	20–10–31	59	17·4	5·6	3·6	305	0·64
2	27–10–31	68	17·7	7·2	4·5	338	0·62
3	2–11–31	64	23·9	13·2	7·9	494	0·60
4	10–11–31	65	31·4	24·5	9·6	834	0·39
5	17–11–31	77	22·1	15·9	8·6	488	0·53
6	20–11–31	60	25·2	19·6	9·4	829	0·48
7	24–11–31	66	21·6	12·7	8·4	806	0·67
8	30–11–31	64	18·5	10·1	5·4	422	0·54
9	7–12–31	68	22·8	15·5	10·0	907	0·64
10	15–12–31	64	21·1	10·1	4·0	781	0·39
11	22–12–31	67	17·6	6·4	3·4	485	0·53
12	29–12–31	73	24·1	14·0	7·2	712	0·51
13	6–1–32	77	18·5	9·5	7·4	682	0·78
14	12–1–32	82	17·1	7·9	7·5	772	0·95
15	20–1–32	78	15·8	5·9	6·2	532	1·04
16	28–1–32	80	15·5	7·6	7·2	582	0·96
17	2–2–32	81	16·3	7·6	6·7	516	0·89
18	9–2–32	73	24·4	15·0	7·8	798	0·52
19	17–2–32	66	29·5	18·5	12·4	817	0·68
20	14–9–32	63	31·9	21·1	12·0	667	0·57
21	14–7–33	57	39·5	16·0	7·0	526	0·44
22	24–7–33	53	38·4	14·9	6·0	1331	0·40
23	28–7–33	56	42·7	14·1	6·3	—	0·45
24	3–8–33	50	41·6	22·7	9·6	—	0·42
25	8–8–33	65	39·0	21·9	10·0	—	0·46
26	12–8–33	54	36·1	21·1	9·7	—	0·46
27	16–8–33	56	38·5	20·0	7·6	—	0·38
28	23–8–33	52	37·1	18·0	6·0	—	0·33
29	28–8–33	59	31·2	19·3	7·0	—	0·36
30	1–9–33	64	33·9	21·3	7·2	—	0·34
31	9–9–33	62	30·5	15·8	6·0	—	0·38
32	12–9–33	54	32·4	19·1	7·0	—	0·36
33	18–9–33	68	29·4	14·7	6·7	949	0·46
34	22–9–33	60	20·5	10·3	5·1	541	0·50
35	25–9–33	59	32·2	25·6	5·5	762	0·21
36	29–9–33	61	32·6	19·7	7·1	794	0·36
37	3–10–33	65	38·6	—	—	929	—
38	6–10–33	61	34·3	—	—	715	—
39	9–10–33	67	32·5	—	—	816	—
40	13–10–33	62	29·1	—	—	613	—
41	16–10–33	65	27·6	—	—	658	—
42	20–10–33	83	24·2	11·5	5·5	475	0·48
43	24–10–33	75	19·1	6·6	4·6	426	0·70
44	27–10–33	61	32·8	18·3	6·4	871	0·35
45	30–10–33	60	30·8	13·6	6·1	603	0·45
46	3–11–33	61	25·5	13·8	4·4	664	0·32
47	7–11–33	61	35·6	—	—	776	—
48	10–11–33	61	35·0	26·4	6·7	662	0·25
49	14–11–33	68	28·8	19·7	7·2	730	0·37
50	17–11–33	72	29·3	16·3	6·9	792	0·43
51	20–11–33	64	32·5	18·7	7·5	978	0·40
52	24–11–33	68	33·9	22·2	8·9	817	0·40
53	28–11–33	71	33·3	18·3	7·5	824	0·41
54	1–12–33	70	31·8	20·2	8·4	997	0·42
55	5–12–33	73	25·5	10·8	5·9	679	0·54
56	8–12–33	79	26·2	12·7	5·6	582	0·44

[1] Average temperature of the day.
[2] In dry soil.
[3] Millions per gm. of oven-dry soil.
[4] Thousands per gm. of oven-dry soil.

We have little definite knowledge concerning the influence of moisture and temperature on the numbers of soil microorganisms. Fabricius and v. Feilitzen (1905) found bacterial numbers in peat soil closely connected with the temperature, but gave few data. Engberding (1909) thought to have established a definite positive correlation between numbers of bacteria and moisture content of the soil; however, a calculation of the correlation coefficient from the data in his tables 5 and 6 shows $r = 0{\cdot}479$ and $0{\cdot}427$, respectively, which cannot be considered significant with only 16 observations (Fisher, 1930, Table V, A). The data of Waksman (1916) did not reveal any correlation of the bacterial numbers with either moisture or temperature. Another series of data published by Waksman (1922), representing 4 counts from each of 10 differently fertilized soil plots, shows in most cases a strong depression of bacterial numbers at low moisture content, but his data are too few for a statistical examination. The same applies to the data of Cobb (1932). Conn (1912) observed a striking parallelism between moisture and numbers of bacteria (including actinomycetes) in two soil plots, but since the samples were taken from two different parts of each plot and the uniformity of the distribution of the bacteria over the plots is unknown, we cannot calculate the correlation. Cutler, Crump and Sandon (1923) did not find the bacterial numbers correlated with either moisture or temperature, like Thornton and Gray (1930), who, however, in one series observed a definite correlation between rainfall and numbers of bacteria. Neither is any such correlation clearly observable in the counts of Smith and Worden (1925).

As to the influence of moisture on the numbers and activities of soil actinomycetes, it is sometimes alleged that these organisms are most active in comparatively dry soils, but very little experimental evidence is available, since the data on this point are but few. The data given by Conn (1912), Waksman (1922), and Cobb (1932) do not show any distinct preponderance, absolute or relative, of actinomycetes over bacteria at low degrees of moisture. Perhaps the strongest evidence for the preference of actinomycetes for dry soil is given by Dubos (1928), who found that cellulose-decomposing actinomycetes displayed their strongest activity at a degree of moisture considerably below the optimum for bacteria and fungi. There is some evidence that the actinomycetes as a whole are less tolerant towards low temperatures than many bacteria (Lochhead, 1926), but otherwise very little is known about their relation to temperature.

Neither do we possess much knowledge concerning the influence of moisture and temperature on soil fungi, so far as their numbers are concerned. Some data published by Waksman (1924) show no correlation whatever between soil moisture and fungal numbers. Cobb (1932) found a depression of fungi at periods of drought, but her results are too few for a statistical examination, and the same applies to the data of Dixon (1928–30), who found the fungal numbers correlated with temperature rather than with moisture.

The results of the present experiments are shown in Table 3, and the correlation coefficients calculated herefrom in Table 4.

Numbers of Bacteria.—As Text-figure 1 shows, there is a very definite correlation between moisture content and bacterial numbers, represented by a correlation coefficient of $0{\cdot}773$; this value still remains very high when the influences of temperature and of actinomycetes are eliminated, but the temperature shows no correlation at all with the bacterial numbers, when the influence of moisture is eliminated. The disagreement of this result with those of Cutler, Crump and Sandon (1923) and Thornton and Gray (1930) is probably due, partly to the less selective character of the counting medium which does not show the marked diurnal changes in the bacterial numbers, partly to the fact that the changes in

TABLE 4.

Correlation Coefficients from Periodical Counts.

Correlation between	r	n	P	Significance.
Bacteria and Moisture, total	0·773	48	<0·01	Positive.
Same, partial, with elimination of:				
Temperature	0·700	47	<0·01	—
Temperature and Actinomycetes	0·638	46	<0·01	—
Bacteria and Temperature, total	−0·493	48	<0·01	Positive.
Same, partial, with elimination of:				
Moisture	0·024	47	>0·1	Negative.
Moisture and Actinomycetes	−0·113	46	>0·1	—
Actinomycetes and Moisture, total	0·324	48	0·05–0·02	Positive.
Same, partial, with elimination of:				
Temperature	0·385	47	<0·01	—
Temperature and Bacteria	−0·075	46	>0·1	Negative.
Actinomycetes and Temperature, total	−0·051	48	>0·1	Negative.
Same, partial, with elimination of:				
Moisture	0·224	47	>0·1	—
Moisture and Bacteria	0·248	46	0·1–0·05	—
Bacteria and Actinomycetes, total	0·567	48	<0·01	Positive.
Same, partial, with elimination of:				
Moisture	0·528	47	<0·01	—
Temperature	0·624	47	<0·01	—
Moisture and Temperature	0·628	46	<0·01	—
Fungi and Moisture, total	0·588	44	<0·01	Positive.
Same, partial, with elimination of Temperature	0·577	43	<0·01	—
Fungi and Temperature, total	−0·208	44	>0·1	Negative.
Same, partial, with elimination of Moisture	0·158	43	>0·1	—
Bacteria and Fungi, total	0·527	38	<0·01	Positive.
Same, partial, with elimination of:				
Moisture	0·118	37	>0·1	Negative.
Temperature	0·500	37	<0·01	Positive.
Moisture and Temperature	0·113	36	>0·1	Negative.
Actinomycetes and Fungi, total	0·432	38	<0·01	Positive.
Same, partial, with elimination of:				
Moisture	0·317	37	Near 0·05	Doubtful.
Temperature	0·456	37	<0·01	Positive.
Moisture and Temperature	0·282	36	0·1–0·05	Negative.
Ratio Act.: Bact. and Moisture, total	−0·692	48	<0·01	Positive.
Same, partial, with elimination of Temperature	−0·492	47	<0·01	—
Ratio Act.: Bact. and Temperature, total	0·607	48	<0·01	Positive.
Same, partial, with elimination of Moisture	0·283	47	Near 0·05	Doubtful.
Moisture and Temperature, total, as applied to Bacteria and Actinomyctes	−0·653	48	<0·01	Positive.
Same, as applied to Fungi	−0·538	44	<0·01	Positive.
Same, as applied to Bacteria, Actinomycetes and Fungi	−0·534	38	<0·01	Positive.

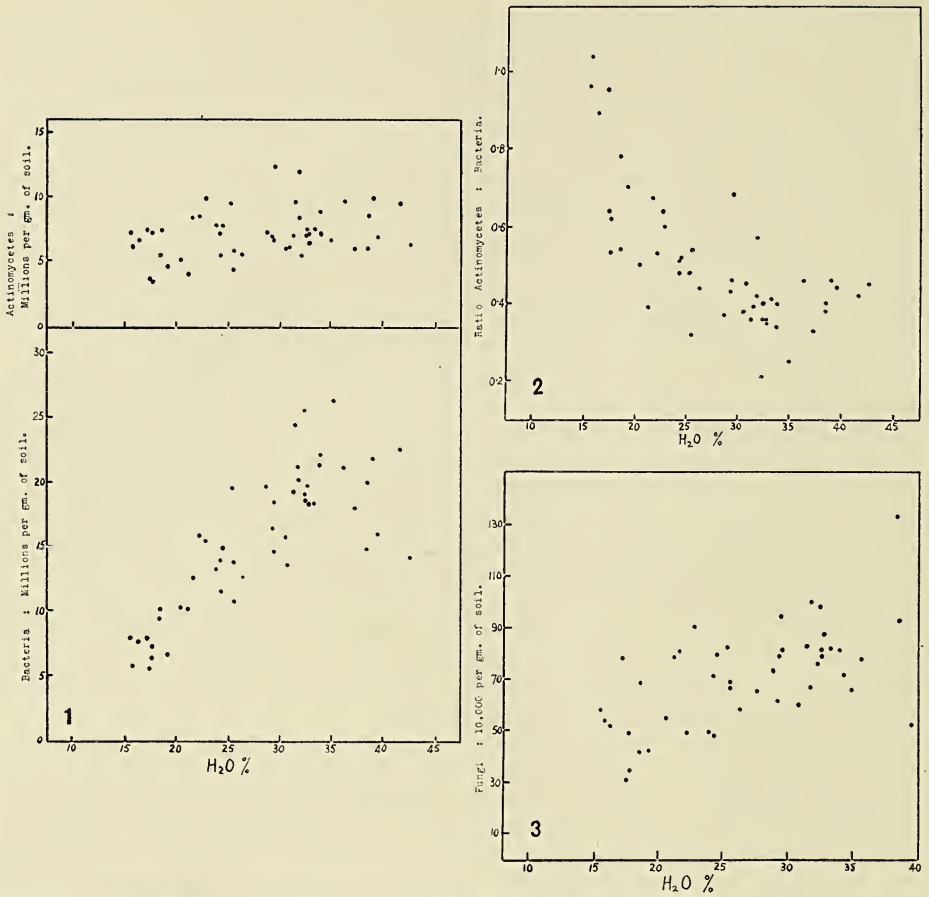
moisture here appear more drastic than in the English soils. The maximal numbers seem to occur at about 32–35% H_2O, which corresponds to 65–70% of the water-holding capacity of this soil, and the numbers seem to have a tendency to decrease at still higher degrees of moisture (40–42% H_2O), but the observations at these high degrees of moisture are too few to enable us to say definitely whether a real optimum exists at 32–35% H_2O, or whether the numbers of bacteria merely here show stronger fluctuations due to other causes. Indeed, if we calculate the correlation coefficient between bacteria and moisture up to 37·1% H_2O, which appears to mark the endpoint of the optimal zone, we find $r = 0{\cdot}882$, a value which is not significantly higher than the value $r = 0{\cdot}773$ applying to the whole range of moisture (Fisher, 1930, p. 170). Since thus the two samples show the same correlation, we cannot draw definite conclusions as to a decrease in bacterial numbers at the highest degrees of moisture observed here.

Numbers of Actinomycetes.—This group of organisms shows some interesting relationships. Their correlation with the moisture, total or with temperature eliminated, is significant, although low (cf. Text-figure 1), but when the influence of the bacteria is excluded, the correlation disappears altogether. Their correlation with the temperature is not significant, but they show a very definite correlation with the numbers of bacteria, particularly when moisture and temperature are eliminated. Whether this is due to an actual stimulation of the actinomycetes by the bacteria, or to some unknown factor that stimulates both groups of organisms simultaneously, remains an open question, but it is worth noticing that actinomycetes play an important role in the decomposition of dead microbial matter in the soil (Waksman and Skinner, 1926; Jensen, 1932), so that we might expect an increase in actinomycetes at periods when bacterial numbers run high and much bacterial protoplasm is being produced.

It should here be remarked that the term "actinomycetes", here as well as in the previous series of experiments, covers the genera *Actinomyces* and *Micromonospora* together with those forms of *Proactinomyces* that produce colonies of a visibly mycelial character. The distinction from the bacteria is thus somewhat arbitrary (the actinomycetes are, botanically speaking, a group of bacteria), but the genus *Actinomyces* accounts for an overwhelming majority of the soil "actinomycetes" (Jensen, 1931*c*).

As a natural consequence of the comparative independence of the actinomycetes on moisture, the ratio of actinomycetes to bacteria shows a strong negative correlation with the moisture content (see Text-figure 2), also if the influence of temperature is eliminated (Table 4). The correlation of this ratio to temperature seems to be positive, but only just on the verge of significance, when the influence of moisture is eliminated. This leads us to the conclusion that the relative abundance of actinomycetes, but not their actual numbers, is largely governed by the moisture supply, perhaps also by the temperature, the actinomycetes being most predominating under conditions of low moisture and high temperature.

Numbers of Fungi.—The correlation between moisture and numbers of fungi (Text-figure 3, and Table 4) is highly significant, although not so close as in the case of the bacteria, and it remains so when we eliminate the influence of temperature, which, on the other hand, shows no significant correlation with the fungal numbers. (It is true that the "numbers of fungi", as determined by plate counting, do not express the numbers of fungus-individuals as they exist in the soil, due to the breaking-up when the soil suspension is prepared, but still



Text-fig. 1.—Numbers of bacteria and of actinomycetes plotted against moisture-content of soil.

Text-fig. 2.—Ratio of actinomycetes to bacteria plotted against moisture-content of the soil.

Text-fig. 3.—Numbers of fungi plotted against moisture-content of the soil.

the fact that the plate-counted numbers are significantly correlated with such definite soil characters as reaction and contents of organic matter and moisture, indicates that they cannot be mere chance figures depending on the fortuitous disintegration of the mycelia and scattering of the spores, but must have a real bearing on the density of mycelia plus spores in the soil.) There is no correlation between fungal and bacterial numbers when the influence of moisture is excluded, and also the correlation between fungi and actinomycetes, with elimination of moisture and temperature, is too low to be considered significant. All in all, there is thus no evidence that these three groups of microorganisms, *per se*, exert any antagonistic influence upon each other, such as is the case with the bacteria and the protozoa, especially the free-living amoebae (Cutler, Crump and Sandon, 1923).

Finally, it is to be noted that no distinct seasonal changes in the numbers, apart from results from the changes in the moisture content, are noticeable in any of the groups of microorganisms. The disagreement of this result with observations made in some other geographical regions (see Thornton and Gray, 1930, and Waksman, 1932) is probably due to the climatic conditions being different from those obtaining in Europe and North America.

Conclusions.

We have now seen, firstly, that the numbers of all the three groups of microorganisms, and particularly the bacteria, increase markedly with increase in the content of organic matter, without being so much influenced by the reaction which, however, largely governs the ratio of fungi to bacteria plus actinomycetes, and secondly, that in a given soil the numbers of bacteria and fungi increase with increasing moisture without being influenced by the temperature (all, of course, within the limits of the present observations), and that the relative abundance of actinomycetes in proportion to bacteria increases strongly with decreasing moisture and probably also with increasing temperature. These results appear interesting in connection with certain aspects of the humus problem. It has been shown (Jenny, 1929–31, and several authors quoted by him) that climatic conditions exert a marked influence on both the quantity and the quality of soil organic matter, the quantity of which increases with decreasing temperature and with increasing humidity, while the carbon : nitrogen ratio becomes narrower with increasing temperature, i.e., the soil organic matter becomes richer in nitrogen, which facilitates the formation of nitrates (Waksman, 1932). The mother substances of soil "humus" are largely lignin and certain proteid compounds possibly derived from the dead bodies of microorganisms, particularly fungi. Actinomycetes are credited with the power of decomposing lignin, which is but very incompletely decomposed at low temperatures (Waksman and Gerretsen, 1931). Therefore we should indeed expect to find a more complete decomposition, i.e., less accumulation of humus with a higher nitrogen content (since lignin is a nitrogen-free compound) under conditions where actinomycetes preponderate over bacteria and fungi, namely, under conditions of low moisture and high temperature.

Summary.

Counts of microorganisms in 50 soils from New South Wales showed a definite correlation between the content of organic matter and the numbers of bacteria, actinomycetes and fungi. This correlation was most pronounced in the case of bacteria, least in the case of actinomycetes. The soil reaction had not in itself any influence on the numbers of bacteria and actinomycetes, but showed a significant correlation with the numbers of fungi, although its influence seemed less marked than that of the organic matter. The ratio of fungi to bacteria plus actinomycetes was distinctly correlated with the reaction, except in the case of certain soils abnormally poor in bacteria and actinomycetes. The moisture-content of the soil did not in this series of observations show any definite influence on any of the groups of microorganisms, but it was noted that several soils from dry districts were very poor in fungi.

Periodical counts of microorganisms in a soil from Sydney showed a strong positive correlation between moisture-content and numbers of bacteria, and a similar, although less pronounced, correlation between moisture and numbers of

fungi, whereas the numbers of actinomycetes did not seem actually to be influenced by changes in the moisture-content. None of the groups of organisms showed any actual correlation with the temperature, or any definite seasonal changes apart from those resulting from the changes in moisture. There was a definite positive correlation between the numbers of bacteria and actinomycetes, apart from the effects of moisture and temperature. The ratio of actinomycetes to bacteria became wider with increasing moisture, and there was also a certain evidence that this ratio becomes narrower with increasing temperature, i.e., the actinomycetes tend to predominate under conditions of low moisture-content and high temperature.

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